Pythium and *Phytophthora* associated with root disease of hydroponic lettuce

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DECLARATION

The work presented in this thesis is original and contains no material formerly published or written by another person, except where due acknowledgement has been specified. I hereby declare that I have not submitted this material to any institution for a degree or diploma.

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SUMMARY

Root rot disease of lettuce grown hydroponically has become a serious problem in Australia and worldwide. Farmers in Australia claim that they have suffered heavy yield losses of hydroponic lettuce in summer in recent years. The research reported in this thesis focused on root disease of lettuce grown in hydroponic systems in the Sydney area and included determination of the causes of this disease, isolation of pathogenic organisms and identification of pathogens by using morphological and physiological studies, as well as molecular techniques. The technique involving Inter Simple Sequence Repeats (ISSR) was also used to study the relationships among the populations of these pathogens. Moreover, the effect of the temperature of the nutrient solution on disease development was also investigated. The research reported here represents the first comprehensive survey of hydroponic lettuce farms in and near Sydney, New South Wales (NSW) in relation to root disease.

Two surveys investigated root disease severity on lettuce grown in hydroponic systems in the Sydney and Central Coast areas of NSW. Three different lettuce cultivars (Baby Cos, Red Oak and Brown Mignonette) were surveyed five times over an 11 month period (May 2003 to March 2004) in one farm (Leppington 1) in the first survey. In the second survey, four different lettuce cultivars (Baby Cos, Red Oak, Green Oak and Brown Mignonette) were surveyed five times over an 11 month period (May 2004 to March 2005) in four different farms (Leppington 1 and 2 and Central Coast 1 and 2). From these two surveys, it appears that root disease of hydroponic lettuce occurred at farms only in the warmer times of the year, when the nutrient solution temperature was 20-30.5°C, and not in the cooler times of the year, when the nutrient solution temperature was 13.5-18°C.

In order to isolate pathogenic organisms, lettuce plants were sampled during the two farm-based surveys. Isolations were carried out from the roots of the cultivars surveyed from the same farms and at the same times as the surveys. Two genera of oomycetes, *Pythium* (81 isolates) and *Phytophthora* (68 isolates), were the main microorganisms isolated from lettuce roots grown in hydroponic systems. *Pythium* was isolated all year round (from 60-100% of samples), but a disease problem at the farms only occurred in

the months with higher temperatures (November, January and March). *Phytophthora* was isolated nearly all year around (from 19-80% of samples).

Isolates of *Pythium* and *Phytophthora* were generally found to be pathogenic to lettuce plants at 25°C and 35°C, but not at 15°C, when lettuce were grown in potting mix. *Pythium coloratum* was found to be pathogenic to lettuce plants grown in an experimental hydroponic system when the nutrient solution temperature was between 22°C and 26°C. Other fungi, such as *Fusarium* spp. and *Rhizoctonia* spp., were also isolated but only infrequently and they were not associated with root disease in the farm at the time of isolation. Furthermore, they were not pathogenic to lettuce grown in potting mix at 15, 25 or 35°C.

The effects of the temperature of the nutrient solution on root disease of lettuce caused by *Pythium* and *Phytophthora* were examined in an experimental hydroponic system. Root rot disease occurred following inoculation with an isolate belonging to *Pythium* group F, or a combination of this isolate and *Phytophthora drechsleri*, under a temperature regime of 24-27°C but not at 16-17°C. Yield reduction was found in plants inoculated with an isolate belonging to *Pythium* group F, *Phytophthora drechsleri* and a combination of the two, at a nutrient solution temperature regime which involved exposure to 34°C for 10 hours, followed by 18-20°C for the remainder of the experiment.

Morphological features and physiological characteristics were used to identify 81 isolates of *Pythium* and 68 isolates of *Phytophthora* obtained from roots of hydroponic lettuce. Molecular techniques were also used for identification including polymerase chain reaction-random fragment length polymorphisms (PCR-RFLP) and sequencing of the internal transcribed spacer (ITS) region of rDNA. For population studies, the ISSR technique was used. The 81 isolates of *Pythium* could be divided into three groups on the basis of colony characteristics. Eighty *Pythium* isolates were identified as belonging to *Pythium* group F and one isolate as *Pythium coloratum*. All 68 *Phytophthora* isolates were identified as *Phytophthora drechsleri*. The optimum growth temperature of the isolates belonging to *Pythium* group F and the isolate of *Pythium coloratum* was 30°C. They also grew well at temperatures of 25°C and 35°C and could still grow at 40°C and 5°C. The optimum growth temperature for the isolates of *Phytophthora drechsleri* was

25°C but they were still able to grow at temperatures of 10°C and 35°C. An assessment of mating type was used as a biological marker for all *Phytophthora drechsleri* isolates. All isolates were found to be heterothallic and of the A_1 mating type. They produced oogonia with amphigynous antheridia when paired with the A_2 mating type of *Phytophthora cryptogea.*

When 81 *Pythium* isolates were examined using four primers with the ISSR technique, 11 groups were established. A slight correlation was found between the groups and the sampling times at which isolates in the groups were obtained. However, no correlations were found between the groups and either the farm or the geographic area from which isolates were obtained. Furthermore, there was no correlation between these groups and the lettuce cultivars yielding the isolates. Moreover, no correlations were found between the groups established by the ISSR technique and the three groups identified on the basis of colony characteristics.

The ISSR technique applied to *Phytophthora* isolates yielded six groups. A correlation was found between these groups and the sample times at which isolates were obtained, on the basis of cooler season samples (May and August together) compared with warmer season samples (November, January and March together). No correlation was found between the groups and either the farms or the geographic areas from which the isolates were obtained. Furthermore, there was no correlation between the groups and the lettuce cultivars yielding the isolates.

Based on the findings of this research, root rot disease management in hydroponic lettuce could be achieved by reducing the temperature of the nutrient solution in summer to 20°C or less, whilst maintaining it within a range favourable to lettuce growth. Moreover, methods to reduce the inoculum level of *Pythium* (and possibly *Phytophthora* as well) are worth investigating, as are methods of disease management based on biological control.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BGT	Botanic Gardens Trust
bp	base pair
BSA	Bovine serum albumin
CLA	Carnation Leaf Agar
cm	centimetre
CMA	Corn Meal Agar
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
DI	Disease Index
DNA	deoxyribonucleic acid
dTTP	deoxythymidine triphosphate
EDTA	ethylenediaminetetraacetic acid
g	gram
GLM	General Linear Model
h	hour
ha	hectare
ISSR	Inter Simple Sequence Repeats
ITS	Internal Transcribed Spacer
min	minute
mL	millilitre
mm	millimetre
NFT	Nutrient Film Technique
ng	nanogram
PCA	Potato Carrot Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PPA	Peptone PCNB Agar
PSA	*Phytophthora* Selective Agar
PYSA	*Pythium* Selective Agar
rDNA	ribosomal DNA
RFLP	Restriction Fragment Length Polymorphisms
rpm	revolutions per minute
s	second
SNA	Spezieller Nährstoffarmer Agar
U	Unit
UTS	University of Technology Sydney
UV	Ultraviolet
WA	Water Agar
µL	microlitre
µm	micrometre